

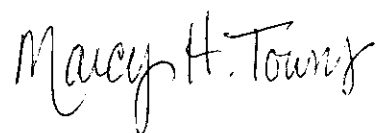
Polymorphism of Sulfathiazole

An Honor's Thesis (HONORS 499)

by

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A handwritten signature in black ink that reads "Marcy H. Towns". The script is cursive and fluid, with the first letters of each name being capitalized and prominent.

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ABSTRACT

This examination of the phenomena of polymorphism, the formation of different crystalline structures within the same chemical compound, is divided into two sections. The theory of polymorphism includes definitions and characteristics of polymorphs, mechanisms and conditions under which polymorphs form, and techniques for identifying and analyzing these polymorphs. The evaluation of a sulfur-containing pharmaceutical compound, sulfathiazole, by differential scanning calorimetry (DSC) for polymorph characteristics will be the focal point of this project. The effects of solvent and temperature, as well as the solubility of each compound, will be observed. Graphical data will be included to illustrate the transformations and melting points of each polymorph.

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2. INTRODUCTION

Polymorphs are molecules with the same chemical formula having different crystalline forms. The differences in crystalline forms have been known to occur as functions of solvent characteristics and thermodynamic aspects (enthalpy and free energy), although scientists have been unable to determine the exact nature and causes of polymorph formation within these parameters. Polymorphs are one of the many solid state characteristics a compound can have. "Solid state" simply refers to the phase of the drug compound (solid).

Polymorphs of the same compound can have varying melting points and solubilities. A slight change in solubility can affect the bioavailability (uptake) of a compound in the human body, so not coincidentally the evaluation of medicinal compounds for polymorphism is extremely important to the pharmaceutical industry. The multiple polymorphs of sulfathiazole (STZ) all have unique melting temperatures. Several polymorphs within one sample would show multiple transition states and melting points on a graph of temperature (in this case, power) versus time. Transition states occur as a polymorph sample shuffles its molecules to create new crystalline structures.

At least five different polymorphs of STZ have been identified (*1*). The object of this work was polymorph screening of a model compound, STZ. The work consisted of studying the solubility of STZ in four different solvents, and recrystallizing it under different conditions (as a function of temperature). Various polymorphs of STZ were recrystallized. The crystals were analyzed using differential scanning calorimetry (DSC).

3. THEORY

3.1 POLYMORPH SCREENING

When a new drug is synthesized and submitted for approval to the Food and Drug Administration (FDA), the submitted application must contain some information on the solid state characteristics of the compound (2). Since the FDA does not regulate polymorph screening, many different techniques can be used to fulfill this requirement. The FDA does expect, however, that many tests using different solvents, concentrations, temperatures, and pH levels be performed on the compound prior to submission. Each polymorph discovered is subject to extensive physical and chemical property evaluations.

3.2 SOLID STATE

3.2.1 Amorphous and Crystalline Solids

Solid pharmaceutical compounds exist in three forms: polymorphs, solvates, or amorphous forms (3). Amorphous compounds have no overall order to their molecular arrangement. Crystalline forms (those having an ordered structure and lattice within the molecule) of drug compounds are polymorphs and solvates.

3.2.2 Polymorphs, solvates, and hydrates

The ability of a substance to form more than one type of crystal is defined as polymorphism. The recognition of this phenomenon is especially important in the pharmaceutical industry, since one polymorph of a compound may prove to be a more

potent drug than any other crystalline form. Polymorphs exhibit the same chemical structure but different chemical and physical features; these characteristics are derived from the various crystal formations. Identification of a useful polymorph with revealing qualities is crucial to the development of an effective pharmaceutical drug.

A pseudopolymorph forms when a molecule of solvent imbeds itself in the crystal structure (4). The pseudopolymorph is called a hydrate when the solvent is water.

Pseudopolymorphs have variations in their physical and chemical properties similar to that in normal polymorphs.

Two categories of polymorphism define the types of crystals that form (3).

Conformational polymorphism occurs when a relatively flexible molecule folds upon itself to form different arrangements; these arrangements subsequently bundle into crystals. Packing polymorphism happens when rigid molecules collect together to form three-dimensional crystals through intermolecular interactions. These interactions are defined as electronic contacts and hypothetical bonding between atoms of two or more molecules.

3.2.3 Solid State Properties described by polymorphism

Structural, chemical, electrical, mechanical, and dimensional solid state properties can affect the formation of polymorphs in drug compounds (4). The various effects of these properties add to the problem of identifying the main cause of polymorph formation in each drug sample.

3.3 FORMATION OF CRYSTALS

3.3.1 Definitions

The types of crystals that form in a given solvent are dependent on the solubility of the compound (5). Solubility of a solid substance (the solute) is defined as the concentration of a solution where the solution phase is in equilibrium with the solid phase at a given temperature and pressure (3). This means that the solid is no longer dissolving, but it also is not recrystallizing.

Solutions can be described in three ways (3). Undersaturation describes solutions where more solute is able to be dissolved. In other words, crystals will continue to dissolve completely in undersaturated solutions. Saturation is the state where the solid is in equilibrium with the solution. Crystals will neither dissolve nor recrystallize in saturated solutions. Supersaturation pertains to solutions that have more solid dissolved in them than the solution can hold. This state can be obtained by dissolving solute in the solvent at high temperatures, and then rapidly cooling the reaction vessel. Solutes may be precipitated from supersaturated solutions if the solution is disturbed, or if seed crystals are added to the solution.

3.3.2 Crystal Growth

Crystal growth begins with nucleation (3). Individual atoms or molecules collide as they randomly move in solution, and eventually amass into a small nucleus. The direction of nucleation can be influenced by seeding. Seeding involves introducing a seed crystal of a

known polymorph into a solution to drive the formation of that specific polymorph. Nuclei then collect more unit atoms or molecules to form crystals, either through conformational or packing polymorphism as described above (4).

3.3.3 Crystal Defects

Defects can occur as a result of the randomness of crystal formation. The defects are defined as sites of imperfection of packing of crystals (4). These defect sites are the most reactive and vulnerable parts of the polymorph. Vacancies in the crystal structure, twists, and edge defects are some of the most common crystal defects.

3.4 POLYMORPHISM OF SULFATHIAZOLE

Sulfathiazole (STZ) has been studied thoroughly for polymorphs, and scientists have begun an extensive exploration of its solvates (5). Even though there has been some debate concerning the number of polymorphs of STZ, it is commonly understood that five polymorphs are produced upon recrystallization from a number of solvents. In Table 1 below the polymorphs of STZ are listed with pertinent information. Melting and transition temperatures are listed according to Kuhnert-Brandstätter (1).

Table 1. Characteristics of STZ Polymorphs

Reference Code	SUTHAZ01	SUTHAZ	SUTHAZ02	SUTHAZ04	SUTHAZ05
Thermal Behavior (M = Melting) (T = Transition to SUTHAZ01)	M at 201°C	T at 150-170°C or 173°C	M at 173°C; T at 150-175°C	----	M at 196°C

3.5 METHODS OF IDENTIFICATION AND ANALYSIS

Many methods of identification and analysis have been developed to evaluate solid state drug compounds (3). X-ray crystallography and x-ray powder diffraction take advantage of the intensity and wavelength of x-rays to examine the internal structures of crystals. Microscopy can provide easy observation of phase changes, three-dimensional views of a crystal, and surface characteristics of solids on a molecular or atomic scale. Infrared spectroscopy (FT-IR) is especially sensitive to structure, environment, and conformation of a compound, and therefore is a wonderful method for analyzing polymorphs. Solid-state nuclear magnetic resonance (NMR) can analyze solids for the spatial arrangement of the carbon atoms in the crystal, as well as the environment of the solid. Thermal analyses such as differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) involve heating the sample and measuring any changes in its physical properties. TGA measures the change in mass of the sample as it is heated; DSC measures the change in enthalpy (heat, or power) to the sample as it is heated (6).

4. EXPERIMENTAL

4.1 MATERIALS AND METHODS

4.1.1 Compounds and Solvents

A sulfur-containing compound, sulfathiazole (STZ), was examined for polymorphism. Four different solvents were used to create saturated solutions of STZ—n-propanol, acetone/chloroform mixture (1:1 by volume), cool water, and hot water. These solvents

were randomly selected for their universal solvation characteristics, as well as their polar nature. This polar character may affect how crystals of STZ form.

Table 2. List of Solvents and Abbreviations (for simplicity in future reference)

SOLVENTS AND MIXTURES USED	
Cool Water (CW)	
Hot Water (HW)	
n-Propanol (N)	
Acetone/Chloroform Mixture 1:1 by Volume (AC)	

Table 3. Physical Properties of Solvents (7)

SOLVENT	MOLECULAR WEIGHT (G/MOL)	DENSITY (G/CM)	MELTING POINT RANGE (°C)	BOILING POINT RANGE (°C)
n-Propanol	60.10	0.78505	-88.5 to -89.5	82.5
Acetone	58.08	0.788	-94.0	56.5
Chloroform	119.38	1.4476	-63.5	61-62
Water	18.01	1.00	-4 to 0	98-100

Sulfathiazole (STZ) ($C_9H_9N_3O_2S_2$)



Figure 4.1.1a. Three-dimensional space-filling diagram of sulfathiazole created on SpartanBuild software (8).

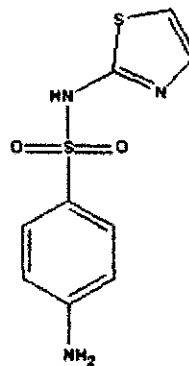


Figure 4.1.1b. One-dimensional diagram of sulfathiazole showing connectivity and double bond arrangement (9).

STZ is one of many drugs classified as sulfonamides, or sulfa drugs. STZ is routinely used to treat bacterial infections, or to inhibit bacterial growth (10).

Table 4. Physical and Chemical Properties of Sulfathiazole (7)

MOLECULAR WEIGHT (G/MOL)	255.32
MELTING POINT RANGE	202-202.5°C
SOLUBILITY	60 mg in 100 mL water at room temperature; also soluble in acetone, mineral acids, KOH and NaOH, ammonium hydroxide
PHARMACEUTICAL PROPERTIES	Antibacterial

4.1.2 Recrystallization Techniques

STZ (Aldrich Chemicals, batch #10316) was recrystallized according to the following procedural synopsis (11):

- (1) n-Propanol: Heat n-propanol to boiling (~95°C). Put approximately 1 gram of STZ in a small beaker, and add just enough hot n-propanol to dissolve completely. Small, white, cylinder-shaped crystals should form as the solution cools. Collect crystals by vacuum filtration.
- (2) Acetone/Chloroform Mixture: Prepare a saturated solution of acetone and STZ. Dilute the solution with an equal amount of chloroform. Small, white crystals will form. Collect them by vacuum filtration.
- (3) Hot Water: Boil a supersaturated solution of STZ in water to dryness using a hot plate. As soon as evaporation is complete, place the white crystals in a hot air oven at 105°C. Take care not to overheat.
- (4) Cool Water: Recrystallize from water. Make a saturated solution, pour off solution into beaker, and place beaker in an ice bath. Collect crystals by vacuum filtration.

4.1.3 Method of Identification and Analysis

STZ crystals were heated in a Perkin-Elmer Pyris 1DSC (serial #537N1032702), and transition states and melting temperatures were recorded by computer software (Pyris Thermal Analysis Software for Windows, Version 4.01, 2001). The DSC apparatus contains a heating element and two pans that hold samples. One pan contains the sample to be examined for polymorphs, along with a reference pan (blank) holding only air. Below is a schematic diagram of the differential scanning calorimeter (DSC).

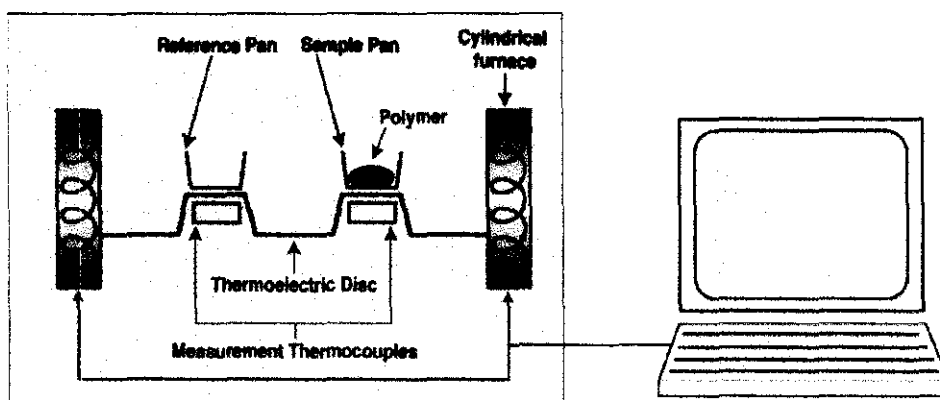


Figure 4.1.3a. Schematic Diagram of Differential Scanning Calorimeter (6).

The DSC attempts to regulate the temperature in each sample as the crystals are heated and melt. This allows for an accurate reading of transition states and melting temperatures (6). Melting is an endothermic process, which means that an input of energy, heat, or power is required for the reaction to occur. The DSC delivered more power to the STZ sample as it melted to keep both sample temperatures the same. STZ was heated from 140°C to 175°C with a rate of two to 10 degrees per minute (10).

The software recorded the increase in power (in milliwatts [mW]) as peaks or endotherms on a graph over time. Sharp peaks indicate melting of crystals, while rounded hills

indicate transition stages. Polymorphs were identified by examining these plots, analyzing the temperatures at which transitions took place, and comparing the data values to the known values listed above in Table 1 under section 3.4.

4.2 RESULTS

4.2.1 Solubility of Sulfathiazole

The solubility of sulfathiazole in the solvents varied. Solubility in water was poor, but somewhat better in hot water than cool water—as was to be expected, since solubility is a function of temperature. STZ dissolved well in acetone; the level of solubility in n-propanol was slightly less than that in acetone/chloroform. There was some resistance of sulfathiazole to continue its level of solubility in the acetone/chloroform mixture once chloroform was added to the mixture.

4.2.2 The Effect of Solvent

The effect of solvent on polymorph formation was dramatic. As Table 5 shows, the types of polymorphs formed varied widely among the solvents used. Please see Thermograms A-D on pages 13-16 for data. Each thermogram displays a different plot of heat flow (mW) versus temperature for each of the samples. The large endotherm at the far right of all graphs indicates the melting point of the most stable compound, SUTHAZ01. Each graph has this same endotherm, indicating that each sample ultimately contains the same compound after all transitions. Interestingly, the plots also contain smaller endotherms. The lesser endotherms at varying temperatures indicate transformations between polymorphs.

Sulfathiazole

Thermograms A-D show the transitions and melting points of STZ. Both graphs A (n-propanol) and B (acetone/chloroform) show strong, even baselines and definite peaks (at 204.295 and 203.461°C respectively), indicating the melting of SUTHAZ01. No transition peaks are shown in either trial of n-propanol or acetone/chloroform.

Thermograms C and D differ from the data given by n-propanol and acetone/chloroform, and also from each other significantly—although they are from the same solvent, water.

Thermogram C (cool water) shows a transition endotherm at 165.642°C and a melting endotherm of SUTHAZ01 at 202.757°C. According to Table 5, the transition endotherm could result from either SUTHAZ or SUTHAZ02 transforming to SUTHAZ01.

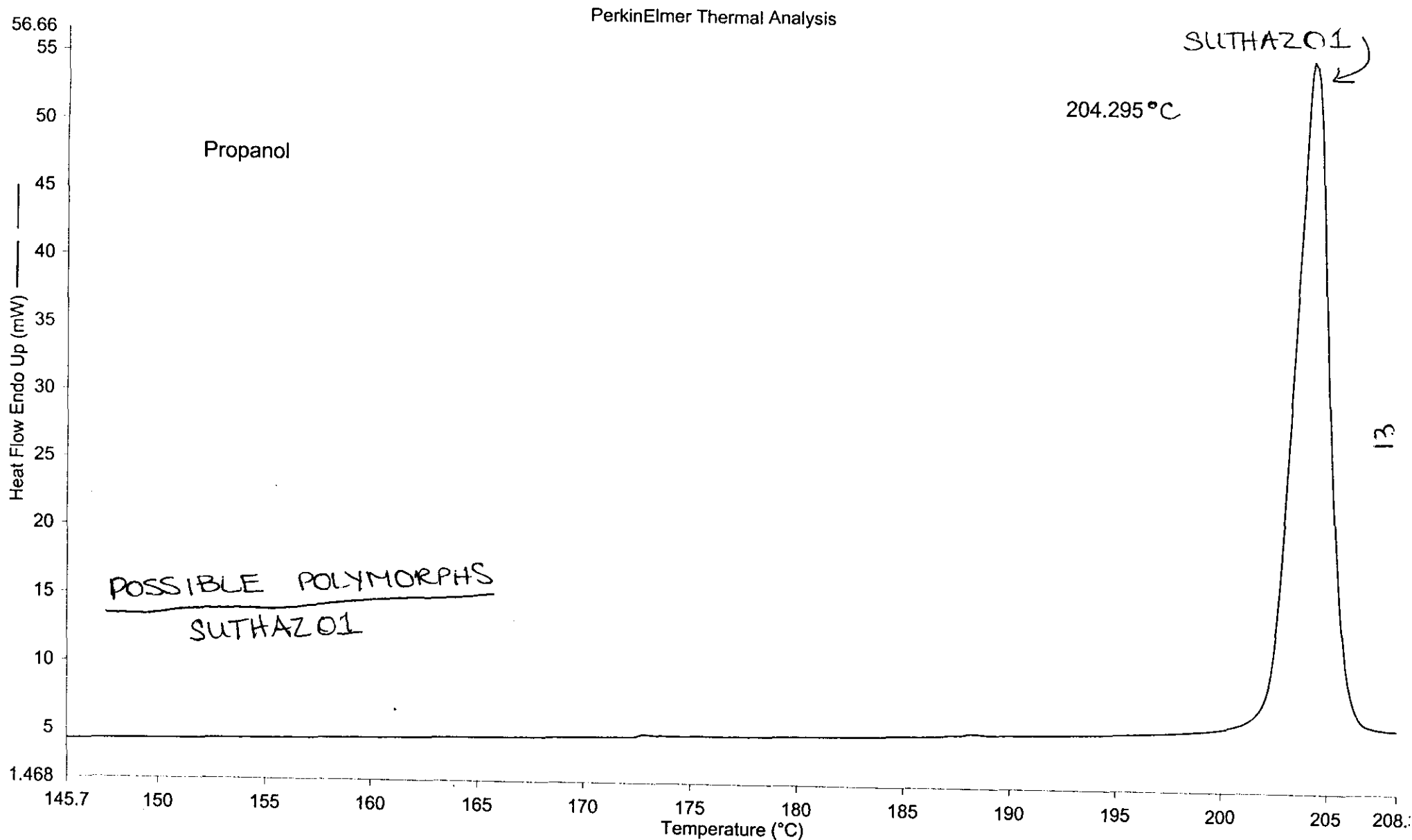
Thermogram D (hot water) best depicts multiple polymorph presence. The small transition endotherm at 161.993°C is most likely the result of the transformation of SUTHAZ or SUTHAZ02. The second, larger endotherm at 199.656°C is peculiar, and indicates the transition of SUTHAZ05. After all transitions have taken place, the melting endotherm of SUTHAZ01 follows at 204.563°C.

Table 5. Thermal Behavior of STZ Samples

GRAPH	1 ST TRANSITION	2 ND TRANSITION	MELTING
A (N-PROPANOL)	None	None	204.295°C
B (ACETONE/CHLOROFORM)	None	None	203.461°C
C (COOL WATER)	165.642°C; from either SUTHAZ or SUTHAZ02 to SUTHAZ01	None	202.757°C
D (HOT WATER)	161.993°C; from either SUTHAZ or SUTHAZ02 to SUTHAZ01	199.656°C; from SUTHAZ05 to SUTHAZ01	204.563°C

Filename:
Operator ID:
Sample ID: n-propanol, 11/13/02
Sample Weight: 1.000 mg
Comment: Ok....I am too good?

THERMOGRAM A



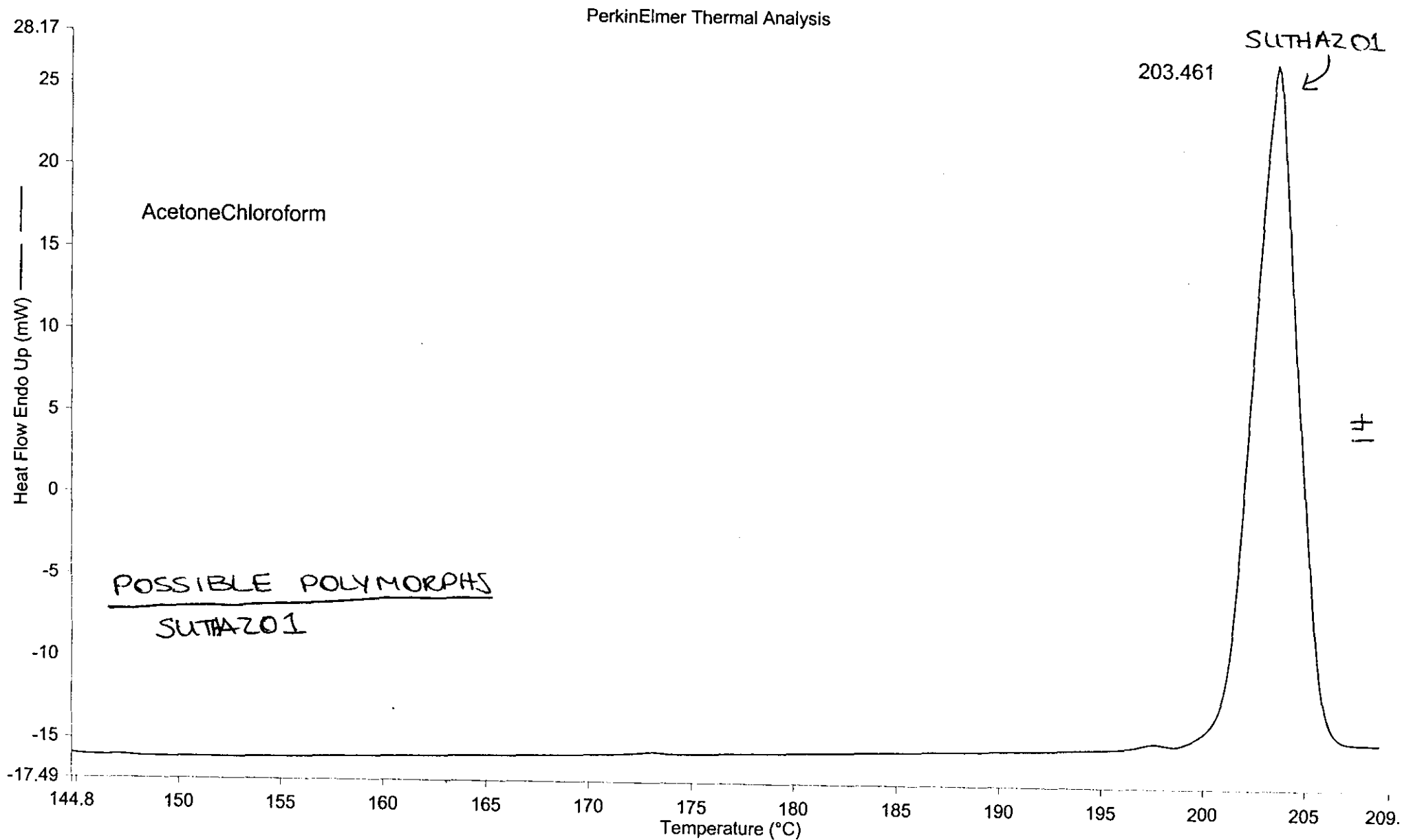
1) Hold for 4.0 min at 140.00°C

2) Heat from 140.00°C to 210.00°C at 7.00°C/min

11/13/2002 3:55:34 PM

Filename:
Operator ID:
Sample ID: acetone/chloroform, 11/13/02
Sample Weight: 1.000 mg
Comment: Dear, God, Help us.

THERMOGRAM [B]



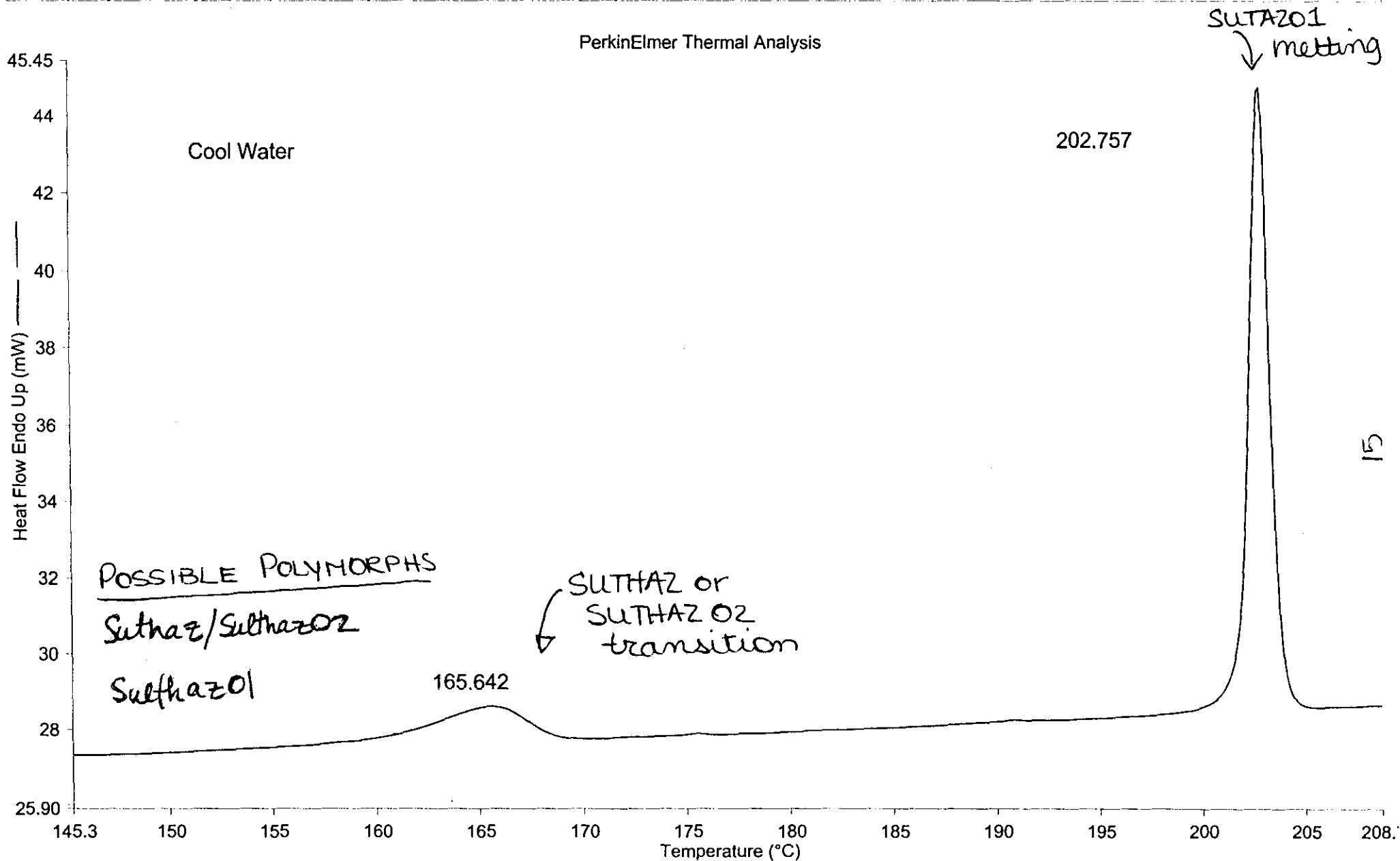
1) Hold for 4.0 min at 140.00°C

2) Heat from 140.00°C to 210.00°C at 7.00°C/min

11/13/2002 3:34:59 PM

Filename:
Operator ID:
Sample ID: cool water, 11/13/02
Sample Weight: 1.000 mg
Comment: It's candy, man.

THERMOGRAM C



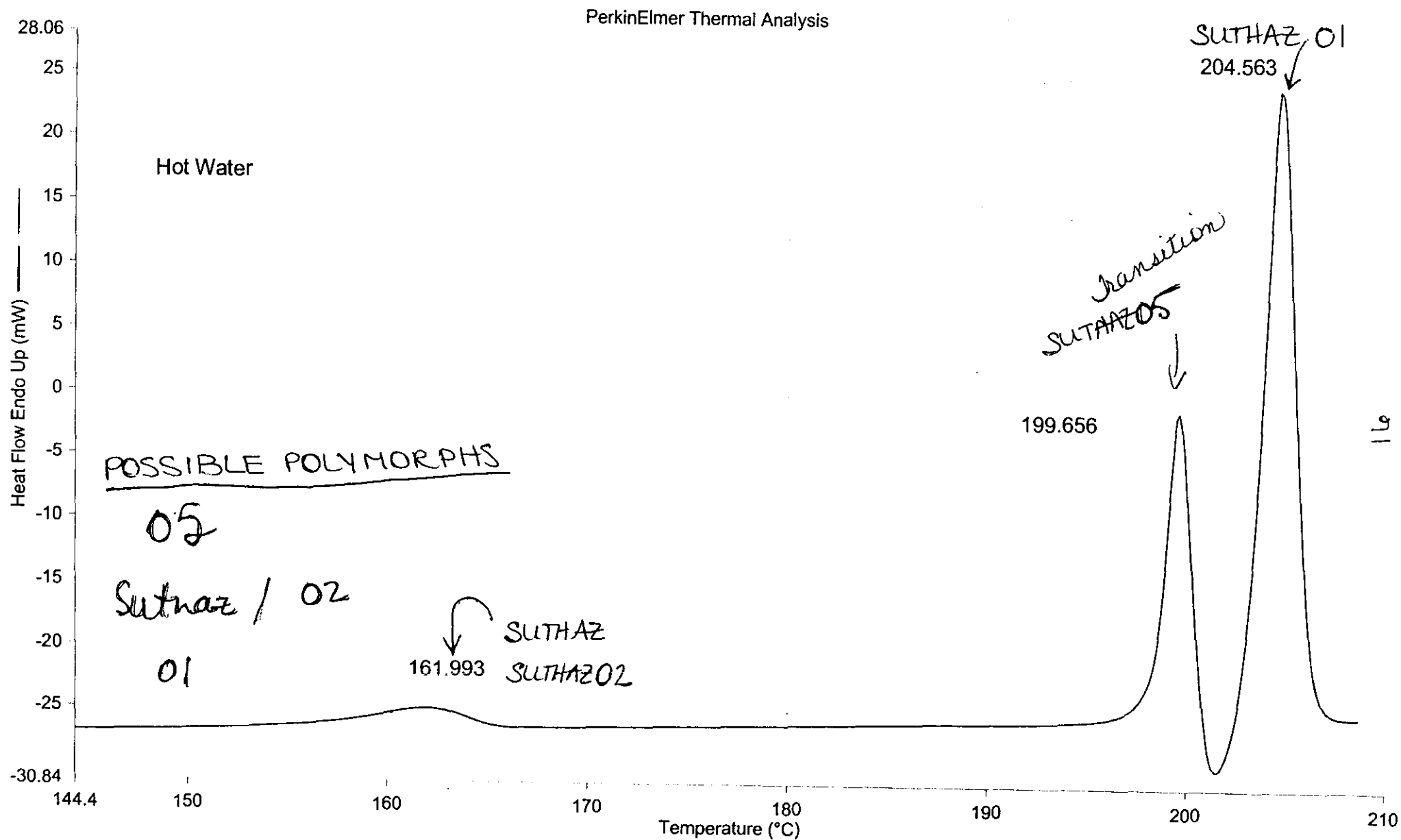
11/13/2002 3:13:17 PM

1) Hold for 4.0 min at 140.00°C

2) Heat from 140.00°C to 210.00°C at 7.00°C/min

Filename:
Operator ID:
Sample ID: hot water, 11/13/02
Sample Weight: 1.000 mg
Comment: good luck

THERMOGRAM D



1) Hold for 4.0 min at 140.00°C

2) Heat from 140.00°C to 210.00°C at 7.00°C/min

11/13/2002 2:59:38 PM

4.1.1 The Effect of Temperature

It is interesting to note that water at different temperatures recrystallized the sulfathiazole samples with different polymorphs. Hot water could have recrystallized up to four different polymorphs: SUTHAZ, SUTHAZ01, SUTHAZ02, and SUTHAZ05. Cold water could have only recrystallized three: SUTHAZ, SUTHAZ01, and SUTHAZ02.

5. CONCLUSION

DSC analysis of sulfathiazole showed that at least 4 polymorphs of STZ exist in recrystallized samples. The organic solvents used in this experiment, n-propanol and acetone/chloroform, produced a single polymorph. Water, at different temperatures, produced varying numbers of polymorphs of STZ. Of the four solvents used, the best polymorph formation was found in the hot water sample. Perhaps the differences in crystalline forms, functioning as elements of solvent characteristics and thermodynamic aspects (change in heat and free energy), as addressed in the Introduction, caused varying polymorph formation within the same solvent.

APPENDIX

Coding Chart of All Experimental Combinations

NST	NSN
ACST	ACSN
CWST	CWSN
HWST	HWSN

XXYY

XX = Solvent (N = n-propanol, AC = acetone/chloroform, CW = cool water, HW = hot water)

YY = Compound (ST = sulfathiazole, SN = sulfanilamide)

Thermograms

Thermogram data is provided on pages 13-16. All transitions and peaks are labeled with the appropriate polymorph identification. Axes of graph: Heat Flow (mW) vs. Temperature (°C).

Thermogram A, p. 13 – Polymorphism of STZ in n-propanol

Thermogram B, p. 14 – Polymorphism of STZ in acetone/chloroform mixture (1:1) by volume

Thermogram C, p. 15 – Polymorphism of STZ in cool water

Thermogram D, p. 16 – Polymorphism of STZ in hot water

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